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A Survey for 32 Nucleotide Deletion in the CCR-5 Chemokine Receptor Gene (Δccr-5) Conferring Resistance to Human Immunodeficiency Virus Type 1 in Different Ethnic Groups and in Chimpanzees

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The 32 nucleotide deletion in the CCR-5 chemokine receptor gene referred to as Δccr-5 has been shown to confer resistance to HIV-1. Using PCR, 1,105 human subjects and 33 common chimpanzees were genotyped attributing them to one of the three possible genotypes: wild-type homozygote (w/w); Δccr-5 homozygote (Δccr-5/Δccr-5) and Δccr-5/wild-type heterozygotes (Δccr-5/w). The ethnic groups investigated included different Middle Eastern nationalities (mainly Arab) and Russians. Carriers of the Δccr-5 mutation were found among Arabs, Iranians and Russians. The highest frequency of the mutation was seen in Russians (24.4% of the Δccr-5 heterozygotes, allele frequency–0.1221). Surprisingly, the only Δccr-5 homozygote identified in our study was an Egyptian. The origin of the Δccr-5 mutation in the Middle Eastern populations, both Arab and non-Arab, is most probably due to a gene flow from the Europeans. The frequency of the Δccr-5 mutation in Russians is one of the highest known. It might be one of the factors contributing to a relatively slow pace of increase in the incidence of sexually acquired HIV infection in Russia. None of the chimpanzees tested was positive for Δccr-5. Interestingly, the DNA sequence of the chimpanzee CCR-5 gene in the region including the site of the Δccr-5 mutation, and flanking areas, was virtually identical to the homologous human sequence, only two mismatches (silent substitutions) were found. ***J. Med. Virol. 55:147–151, 1998.***

KEY WORDS: HIV-1; genetic resistance; PCR; allele frequency

INTRODUCTION

The existence of human beings remaining HIV-free despite repeated exposure to the virus through sexual contacts, so-called 'exposed–uninfected' individuals, is one of the most challenging and intriguing observations in the HIV/AIDS field [Detels et al., 1994; Paxton et al., 1996]. The genetic nature of such resistance has been presumed. However, until recently, proof of this has been lacking and investigations of this phenomenon have been carried out mainly in the framework of descriptive epidemiology. This situation has changed with the identification of the 'second HIV receptor' (also called 'co-receptor'). Two major HIV co-receptors are known. The CXCR-4 chemokine receptor (also called fusin or LESTR) serves as the co-receptor for T-cell line (T) tropic, syncytium-inducing (SI) strains of HIV-1. The CCR-5 chemokine receptor (also designated as C-C CKR-5) functions similarly for macrophage (M) tropic, non-syncytium-inducing (NSI) strains of HIV-1 [Alkhatib et al., 1996; Choe et al., 1996; Deng et al., 1996; Dragic et al., 1996; Doranz et al., 1996; Feng et al., 1996]. There are several other molecules (CCR-3, CCR-2) belonging to the same receptor family which can serve as "facilitators" for HIV-1 entry into target cells, however, their co-receptor activity is significantly lower than that of the CCR-5 and CXCR-4 [Dragic et al., 1996; Doranz et al., 1996; Choe et al., 1996].

The relevance of the HIV co-receptors to genetic resistance to the virus became apparent when an identical homozygous mutation was identified in some 'exposed–uninfected' individuals [Liu et al., 1996]. The mutation, a 32 nucleotide deletion in the CCR-5 gene (Δccr-5), results in truncation of the CCR-5 protein and abrogation of both its normal (mediation of chemokine signaling) and HIV-1 co-receptor functions. As a result the cells bearing Δccr-5 in the homozygous state are resistant to M-tropic HIV-1 in vitro. Importantly, this is not just an in vitro phenomenon—the Δccr-5/Δccr-5

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homozygotes are virtually absent among HIV-1 infected individuals (only one exception is known) [Samson et al., 1996b; Biti et al., 1997]. Most probably the latter is a compounded result of the macrophage resistance to HIV-1 and a predominant role played by M-tropic HIV-1 strains in person-to-person transmission of HIV-1.

One of the important aspects of Δccr-5-related studies is obtaining information on the global, regional and ethnic distribution of this medically important mutation. Unexpectedly, Δccr-5 has been found to be quite common among individuals of West European descent (mutant allele frequency 0.080–0.092). At the same time the mutation has not been observed in individuals of African, South American and Japanese origin [Liu et al., 1996; Samson et al.,1996b]. The frequency of Δccr-5 in other ethnic groups was not known when this study was started.

Data are now presented on the frequency of the Δccr-5 mutation in different Middle Eastern ethnic groups and in Russians. In addition, data are included showing the absence of Δccr-5 in common chimpanzees as well as the close similarity of human and chimpanzee CCR-5 gene sequences in the region encompassing the stretch of nucleotides deleted in the Δccr-5 allele.

MATERIALS AND METHODS

DNA Samples

DNA samples from 1,105 individuals have been tested. The DNA was extracted from leukocytes using the 'Chelex 100' method [Walsh et al., 1991]. The study group, except the Russians, included voluntary blood donors and healthy relatives of individuals referred to the Kuwait Medical Genetics Centre. The DNA samples from the Russians were obtained from patients admitted to the Institute of Pulmonology and Cancer Centre, Moscow. The latter set of the samples was kindly provided by Dr. A. Tatosyan.

DNA samples from 30 common chimpanzees (*Pan troglodytes*) were obtained from captive animals of two colonies (Laboratory of Central Nervous System Studies of the National Institute of Neurological Disorders and Stroke, NIH, USA; Southwest Foundation for Biomedical Research, USA). The chimpanzee DNA samples were kindly provided by Dr. Bruce Johnson and Dr. Jorg Eichberg.

Polymerase Chain Reaction (PCR) for Detection of the Δccr-5

The primers AV-66 (5′-CTGTGTTTGCGTCTCTCC-3′) and AV-67 (5′-CCTGTGCCTCTTCTTCTCA-3′) were used to amplify a 230 bp fragment of the CCR-5 gene including the site of the mutation.The reaction mixture included standard PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl, 1.5 mM Mg_2Cl), 50 μM of deoxynucleoside triphosphates, 10 pmol of each primer and 5 μl of Chelex 100 extracted DNA from leukocytes in a total volume of 25 μl. Thermocyling (94°C, 55°C and 72°C, 30 sec at each temperature, 30 cycles) was performed in a Perkin-Elmer System 9600. Standard precautions to prevent carry-over artifact were strictly followed [Kwok and Highushi 1989]. The detection of the amplimers was carried out by minigel electrophoresis in 1.7% agarose (NA, Pharmacia) and staining with ethidium bromide.

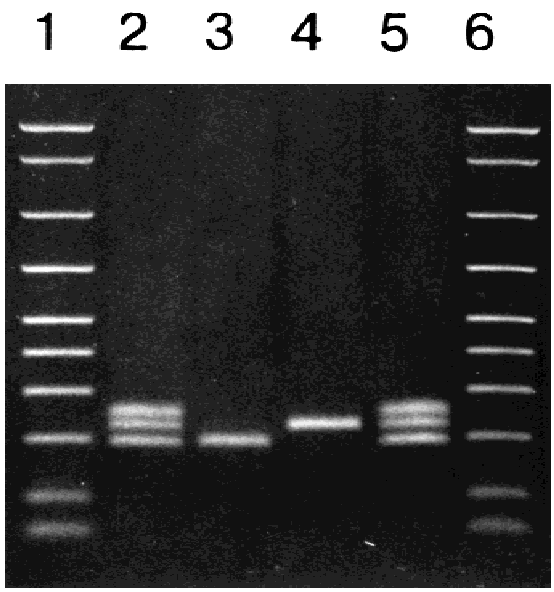

Fig. 1. Typical results of identification of w/w, Δccr5/w and Δccr5/Δccr5 genotypes by PCR. 1, 6–markers (50, 100, 200, 300, 400, 500, 700, 1,000, 1,500 and 2,000 bp); 2 and 5–Δccr5/w heterozygotes (wild type allele band 230 bp, Δccr5 allele band 198 bp, the band above the 230 bp consists of heteroduplexes); 3–Δccr5/Δccr5 homozygote; 4–wild-type (w/w) homozygote.

DNA Sequencing and Analysis

PCR amplified fragments of the human and chimpanzee CCR-5 genes were sequenced directly using AV-66/AV-67 primers according to the manufacturer's (Applied Biosystems/Perkin Elmer Foster City, USA) protocols using Dye Terminator Cycle Sequencing Kit with AmpliTaq DNA Polymerase FS and ABI PRISM 310 Genetic Analyzer. To minimize artifacts due to *Taq* polymerase mistakes, samples were prepared for sequencing as a pool of ten separate PCR amplifications with the same target DNA and primers. DNA sequences were aligned and compared with a help of DNASIS program (Hitachi).

RESULTS

PCR amplification of the region of the human CCR-5 gene defined by the selected primers is expected to produce three possible results, depending upon the genotype of a target DNA: 1) a single 230 bp fragment in the case of a wild-type homozygote (genotype w/w); 2) a single 198 bp fragment in the case of an Δccr-5 homozygote (genotype Δccr-5/Δccr-5); 3) both the 230 bp and 198 bp fragments as well as heteroduplexes in the case of the Δccr-5/ wild-type heterozygote (genotype Δccr-5/w). Strictly in accordance with the expectation we observed three banding patterns (Fig. 1), corresponding to these genotypes. The specificity of the bands repre-

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                          10         20         30         40         50
CCR5- W/W       1 CAGGAATCAT CTTTACCAGA TCTCAAAAAG AAGGTCTTCA TTACACCTGC
CCR-5-Δ/Δ       1 CAGGAATCAT CTTTACCAGA TCTCAAAAAG AAGGTCTTCA TTACACCTGC
CCR-5-PAN       1 CAGGAATCAT CTTTACCAGA TCTCAAAAAG AAGGTCTTCA TTACACCTGC

                          60         70         80         90        100
CCR5- W/W      51 AGCTCTCATT TTCCATACAG TCAGTATCAA TTCTGGAAGA ATTTCCAGAC
CCR-5-Δ/Δ      51 AGCTCTCATT TTCCATACA- ---------- ---------- ----------
CCR-5-PAN      51 AGCTCTCATT TTCCGTACAG TCAGTATCAA TTCTGGAAGA ATTTCCAGAC

                         110        120        130        140        150
CCR5- W/W     101 ATTAAAGATA GTCATCTTGG GGCTGGTCCT GCCGCTGCTT GTCATGGTCA
CCR-5-Δ/Δ     101 -TTAAAGATA GTCATCTTGG GGCTGGTCCT GCCGCTGCTT GTCATGGTCA
CCR-5-PAN     101 ATTAAAGATA GTCATCTTGG GGCTGGTCCT GCCGCTGCTT GTCATGGTCA

                         160        170        180
CCR5- W/W     101 TCTGCTACTC GGGAATCCTA AAAACTCTGC
CCR-5-Δ/Δ     101 TCTGCTACTC GGGAATCCTA AAAACTCTGC
CCR-5-PAN     101 TCTGCTACTC AGGAATCCTA AAAACTCTGC
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Fig. 2. DNA sequence comparison of wild-type and Δccr5 alleles of human CCR-5 gene and homologous sequence of chimpanzee CCR-5 gene. CCR5-W/W–partial sequence of wild-type allele of human CCR-5 gene (prototype sequence of the CCR-5 gene [Samson et al., 1996a], GenBank Accession No. X91492). CCR5-Δ/Δ–partial sequence of Δccr5 allele of the human CCR-5 gene. CCR5-PAN–partial sequence of the chimpanzee CCR-5 gene.

senting the wild-type and the mutant alleles was confirmed by direct sequencing of the 230 bp and the 198 bp DNA fragments. When a common chimpanzee genomic DNA was used instead of human DNA as a target in an otherwise similar PCR test the 230 fragment was also amplified. A partial nucleotide sequence of the chimpanzee fragment (180 nucleotides, the region encompassing the site of the Δccr-5 deletion) is shown in Fig. 2. The sequences of this fragment of human and chimpanzee CCR-5 genes are almost identical (98.9% similarity, only two mismatches; both are silent substitutions). The sequence of the fragment of the chimpanzee CCR-5 gene determined in this study is identical to the sequence of the corresponding region of the chimpanzee CCR-5 gene reported by others [Zacharova et al., 1997].

In total, 1,105 human subjects and 33 chimpanzees have been genotyped. None of the chimpanzees was positive for the Δccr-5 deletion. The results for humans are presented in Table I. The major ethnic group investigated was Arabs (808 individuals). Sixteen of them were heterozygotes and one (Egyptian) was a Δccr-5/Δccr-5 homozygote. Individuals positive for Δccr-5 were not found among Jordanians, Saudi Arabians, Lebanese, Palestinians, Iraqis, Algerians and Omanis. However, the size of these groups was too small to make a reliable estimate of the Δccr-5 frequency in these countries. The average frequency of the Δccr-5 in the Arab populations was 0.0111. The second largest ethnic group investigated was the Russians. The frequency of the Δccr-5 among Russians was found to be very high (0.1221). Among other groups Δccr-5 heterozygotes were found only in Iranians. The frequency of the mutation in this ethnic group (0.0238) was the highest among Middle Eastern populations studied. However, the size of the group was not sufficiently large to conclude that the difference in mutation frequency between Iranians and the Arabs is significant.

TABLE I. Frequency of the Δccr5 Deletion in Different Ethnic Groups

Group	Sample size	Number of Δccr5/w	Number of Δccr5/Δccr5	Δccr5/w allele frequency
Kuwaitis	393	8	0	0.0102
Bedouins	108	4	0	0.0185
Egyptians	72	1	1	0.0208
Syrians	106	3	0	0.0141
Jordanians	52	0	0	<0.0095
Saudis	21	0	0	NA
Lebanese	19	0	0	NA
Palestinians	16	0	0	NA
Iraqis	13	0	0	NA
Algerians	4	0	0	NA
Omanis	3	0	0	NA
Iranians	84	4	0	0.0238
Indians	22	0	0	NA
Filipinos	7	0	0	NA
Bangladeshis	4	0	0	NA
Turks	3	0	0	NA
Sri Lankis	2	0	0	NA
Russians	176	43	0	0.1221

NA, non applicable; allele frequency was not calculated for the small samples (less than 100 chromosomes).

DISCUSSION

Within a few months after the discovery of the Δccr-5 deletion [Liu et al., 1996] it was established that homozygosity for the mutation confers resistance to HIV-1 infection. The resistance to HIV-1 in the Δccr-5/Δccr-5 individuals is profound, though not absolute—

one case of HIV-1 infection in a Δccr-5 homozygous individual has been reported reported in Australia [Biti et al., 1997]. The data on protective significance of the heterozygosity for Δccr-5 are controversial. There are several reports claiming that heterozygosity postpones the progression of asymptomatic HIV-infection to AIDS [Samson et al., 1996; Dean et al., 1996; Huang et al., 1996; Eugen-Olsen et al., 1997; Michael et al. 1997]. On the other hand, at least in one group of AIDS patients (the Danish cohort of HIV-1-positive male homosexuals) the survival of Δccr-5 heterozygotes was significantly shortened [Garred et al., 1997]. In any case, the medical significance of Δccr-5 is unquestionable.

One of the interesting findings reported in the first publications on Δccr-5 was the absence of carriers of the mutation outside Europe and North America. About several hundred non-Europeans, none of them Arab, were tested before the beginning of our study and not a single individual positive for Δccr-5 was found [Liu R et al., 1996; Samson et al., 1996b; Dean et al., 1996]. It has to be mentioned, however, that number of individuals of each ethnic group tested was relatively small and many large ethnic groups have not been tested at all. To close some of the gaps in the knowledge of ethnic distribution of Δccr-5 deletion we undertook a survey of Δccr-5 in Arabs, some Asian ethnic groups and in Russians. Our data clearly shows that the Δccr-5 deletion is not confined to the populations of European descent. The mutation is also present in indigenous Arab and Persian populations of the Middle East, though its allelic frequency in these populations is significantly lower than in Europeans.

There are two possible explanation for the origin of Δccr-5 in the Middle Eastern populations. First, the deletion in the Europeans and in the Arabs arose independently. This seems to be highly unlikely because there are no features in the genomic sequence of the wild-type CCR-5 gene which are suggestive of increased probability of specific deletion in the region of the gene where Δccr-5 is located. The alternative, and more plausible, explanation implies a relatively recent gene flow from Caucasians into indigenous Gulf Arab populations. Importantly, this hypothesis is testable by identification and comparison of haplotypes (defined by polymorphisms in the genomic regions flanking the CCR-5 gene) associated with the Δccr-5 positive chromosomes in Europeans and Arabs.

Another interesting question related to the origin of Δccr-5 is about its timing. Lack of the Δccr-5 in chimpanzees indicates that the mutation arose after the human-chimpanzee lineage split. The absence of the Δccr-5 in indigenous African population indicates that the Δccr-5 arose after migration of pre-historic human beings from Africa (assuming that the "out of Africa" hypothesis is correct). The mutation is clearly much more common among Europeans being probably the commonest among people of East European descent as shown in this study for Russians and as reported by others for Ashkenazi Jews [Martinson et al., 1997]. This is suggestive of a relatively recent origin of the mutation in one of the European ethnic groups. However, before information on the haplotypes of the Δccr-5 positive chromosomes is obtained, the matter remains highly speculative.

The higher frequency of Δccr-5 in the Bedouins residing in Kuwait than in Kuwaiti nationals, and in Arabs on average, deserves attention. The Bedouins belong to different tribes which are highly consanguineous groups. Thus, it is possible that the frequency of the Δccr-5 in some of the tribes may be quite high due to a "founder" effect. Unfortunately, information on the tribal origin of the Δccr-5-positive Bedouins identified in our study was not available.

The only Δccr-5 homozygote identified in the present study was an Egyptian. This was quite unexpected, taking into account that we found only one Δccr-5 heterozygote among 72 Egyptians genotyped. Contamination with Δccr-5/Δccr-5 positive genomic DNA or PCR-amplified fragment can be excluded because the Egyptian sample was the first and the only Δccr-5/Δccr-5 sample present in our laboratory.

While our study was being completed the occurrence of the Δccr-5 in non-Europeans was also reported by Martinson et al. [1997]. These investigators tested, among other ethnic groups, Arabs (241 Saudis and 34 Yemenis). The frequency of the Δccr-5 in Saudis (0.0207) was similar to the findings in Bedouins. None of the Yemenis genotyped by Martinson et al. (1997) was positive for Δccr-5, however, the size of the sample was too small to make a reliable estimate of the mutation frequency.

The frequency of Δccr-5 in Russians is one of the highest known. It has to be mentioned that our sample included mostly Muscovites, most of whom are presumed to be of Slavic origin. However, the ethnic origin of the Russian citizens genotyped in this study was not verified. Interestingly, high frequency of Δccr-5 was also found in other regions of Russia, namely, Udmurtia and Daghestan, which are quite remote from each other and different in ethnic terms [Martinson et al., 1997]. It is tempting to speculate that high frequency of the Δccr-5 mutation could be a factor contributing to the unexpectedly slow growth of the incidence of sexually acquired HIV-1 infections in Russia. However, without a knowledge of the effect of the Δccr-5 heterozygosity on the probability of HIV transmission it is impossible either to prove or to reject this hypothesis.

In conclusion, the Δccr-5 mutation is present in the Middle East, both in Arabs and Iranians, but it is quite rare to influence significantly the pace of HIV-1 spread in the Arab world. The Δccr5 mutation might be one of the factors contributing to the unexpectedly slow pace of the spread of sexually acquired HIV-1 infection in the Russian Federation. In a broader context the knowledge of the distribution of Δccr5 and other mutations influencing outcome of HIV infection [Smith et al., 1997] allows better global and regional modeling of the HIV-1 epidemic as well as prognosis in individual patients. The CCR5 molecule is also a promising target

for new anti-HIV/AIDS therapeutic and preventive interventions which in some cases may have to be tailored according to the Δccr5 status of the individuals.

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